Experimental Studies on the Cooling Irrigation of Cerebral Ventricular System (III) Electron microscopical observation of the choroid plexus and cerebral substance near the ventricular cavity

AUTHOR(S):

NAKAMURA, TERUMI

原　　著

Experimental Studies on the Cooling Irrigation of Cerebral Ventricular System (III)

Electron microscopical observation of the choroid plexus and cerebral substance near the ventricular cavity

by

TERUMI NAKAMURA

From the 2nd Departmant of Surgery, Yamaguchi University, School of Medicine
(Director: Prof. SHUNJI TOKUOKA)

Received for Publication Aug. 2, 1965

INTRODUCTION

Cooling irrigation of the ventricular system has been studied both experimentally and clinically in our department since 1955. Most outstanding feature of the experimental results in this systematical study was acquirement of rapid and reversible unresponsiveness during the irrigation with cold Ringer's solution. In so far as such acquired unresponsiveness, the assumption has been made that the hypothermic effect upon the nervous tissue close to the ventricular wall was responsible for it, from the evidence that the effective hypothermia was obtained only in the restricted layer of a few millimeters from the ependymal surface.

Within the nervous structures, however, it has not yet been clarified whether the blocking of transmission of stimuli took place as a result of organic destruction or functional intermission. The only explanation for denial of organic destruction of the nervous tissues by the cooling irrigation was possibly made on the basis of full reversibility in the acquired unresponsiveness.

Recently, KLAZO[18] and TORACK[33][34] et al. reported that local cooling of the brain brought about localized cerebral edema. According to their suggestion, the ventricular wall and the choroid plexus, both of which may play an important role in interchange of water between blood and cerebrospinal fluid, are probably damaged by hypothermia.

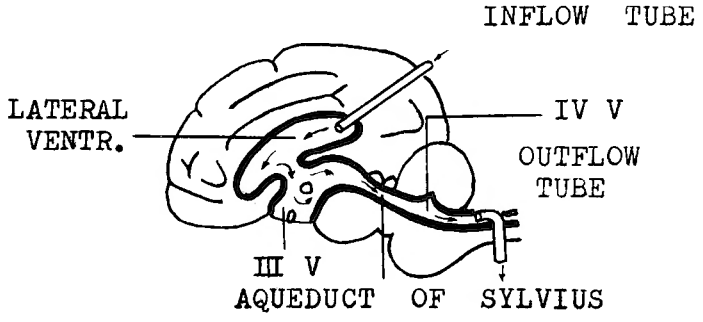
On the other hand, improvement of fixation and advanced technique to obtain ultra-thin section have made the electron microscopical examination easier and more useful. As the ultra-fine structure of nervous tissue has been able to observe, the presence of a unique feature around the capillary such as astroglial process has become to be universally agreed in connection with the anatomical basis of blood-brain barrier, though the morphological evidence of the existence of a structural blood-brain barrier has not yet been confirmed. With the aid of electron microscope, it has also been demonstrated that the basal infolding membrane in the epithelium of the choroid plexus plays an important role in the production

of cerebrospinal fluid.

Those structures above mentioned, such as the ependymal tissue and the choroid plexus are suspected to be subjected to some harmful influence by the procedure of the cooling irrigation because they are in contact with the irrigating fluid. So the morphological investigation of these structures during or after the irrigation has to be carried out. In this paper, electron microscopical findings of these structures following the cooling irrigation of the ventricules are reported.

METHOD

Adult dogs weighing 4 to 7 kg were used. As the basal anesthesia, thiopental sodium was injected intravenously. The method of cooling irrigation of the cerebral ventricles was already reported by TOKUOKA[32] et al. and HIGASHI[16]. The schematic mechanism of cooling irrigation is shown in Fig. 1.



The inflow tube is inserted into the lateral ventricle and outflow tube is put in the major cisterna. The flowing way of irrigating fluid from the lateral ventricle, through the third ventricle, aqueduct of SYLVIUS, and fourth ventricle, to the major cisterna is indicated as arrows.

Fig. 1 IRRIGATING MECHANISM

Twenty nine dogs were used in total, of which six dogs were infused only fixatives which was 1 per cent osmium tetraoxide in acetate-veronal buffer solution with 0.15 M saccharose solution into the ventricles and then the cerebral tissue was taken and fixed again as a control group. The other dogs were all irrigated with 500 ml of cold Ringer's solution and then the specimen were only taken from the dogs which were irrigated successfully. The temperature of the inflow fluid was 6 to 8°C and that of the outflow fluid was 18 to 26°C. The duration of irrigation was about an hour. After cooling irrigation, three dogs were irrigated again with 100 ml of polyvinyl pyrrolidon (P. V. P.), which had been kept at room temperature, at the rate of 10 ml per minute. The other four dogs were irrigated with 100 ml of P. V. P. only at same rates without previous cooling irrigation. After irrigation, the cerebral tissue was excised as soon as possible, but it took about 10 minutes. So as to prevent autolysis of the tissue, 5 ml of fixatives was infused into the ventricle. After the removal of the cerebrum, the choroid plexus and the cerebral tissue around the lateral and third ventricle on irrigated side were cut into small pieces and fixed again. Fixation was carried out by CAULFIELD's[5] method for 20 to 60 minutes. After washing with Ringer's solution, the materials were dehydrated by

passing through ascending alcohol, and then embedded with metacrylate resin. Ultrathin sections were stained by acetic uranyl solution for one to two hours and examined by JEM 5 HS electron microscope.

RESULTS

At the time of removal of the cerebrum, there were no external gross abnormalities; such as swelling, bleeding and cloudiness.

Electron Microscopical Findings of the Cerebral Tissue at the Vicinity of the Ventricles;

In the ventricular wall, the ependymal cells with relatively clear cytoplasm were recognized. The cytoplasm was moderately abundant and near the nucleus fine fibrils which were 75 to 100 Å in diameter was seen characteristically. In the cytoplasm fronting the ventricular cavity, the ciliary shaft was revealed (Fig. 2). Under the ependymal cells, many myelinated nerve fibers were seen and the astrocyte, oligodendroglia and microglia were occasionally recognized. Astrocytes are usually considered to have clear, "watery" cytoplasm with no granular endoplasmic reticulum, relatively few mitochondria and clear processes. However, the nerve cells and the blood capillaries were rarely found too. The intercellular space was very narrow, measuring about 100 to 200 Å.

The capillary blood vessels were surrounded by the clear processes containing a few mitochondria and endoplasmic reticulum. As the clear cell had the abundant cytoplasm of the same density, these clear processes were presumed to be the processes of the astrocyte. The VIRCHOW-ROBIN's space which has been demonstrated in the light microscopic studies was not revealed (Fig. 3). As the electron microscopical alterations of the cerebral tissue near the ventricular cavity after cooling irrigation, enlargement of the clear glial process and swelling of the mitochondria were recognized (Fig. 4). Particularly around the pericapillary space, enlargement of the clear glial process was remarkable and its volume was increased, in consequence of decreased density. However, comparing to electron microscopical observation of the experimental cerebral edema, these changes were relatively mild. Some mitochondria in the clear process located in the pericapillary space were swollen and occasionally recognized near the basement membrane of the capillary blood vessel (Fig. 5, 6).

The capillaries which were surrounded almost completely by the myelinated nerve fibers showed no significant changes. Furthermore, the glial cell, nerve cell and their processes in the other places revealed no alteration. Summarizing above results, cooling irrigation did not cause the dilatation of the intercellular space, but only brought about swelling of the glial processes at the vicinity of the capillary blood vessels. This alteration, however, was relatively slight. The ependymal cell showed no significant change.

Electron Microscopical Findings of the Choroid Plexus;

Electron microscopical findings of the choroid plexus in the lateral and third ventricles were almost the same as those were observed by DEMPSEY and WISLOCKI[8], MAXWELL and PEASE[22] and NAKANISHI[25] in rats, van BREEMAN and CLEMENTE[35], MILLER and ROGERS[23] in rabbits, SHRYOCK and CASE[31] in dogs. Even in low magnification, two peculiar findings were observed in the choroid plexus. The one was the many polypoid

processes which were located in the free surface of the epithelial cells fronting to the ventricular cavity (this surface has been called as the brush border). These processes were demarcated with the cellular membrane which was 70 to 80 Å in width, and their inner area had very low electron density.

The other was that the cellular membrane indented into the cytoplasm even at the basal portion of the epithelium, and this indentation was made of double membrane and its space was almost the same, measuring from 100 to 200 Å in width. MAXWELL and PEASE[22] designated these fine structures as polypoid process and basal infolding membrane respectively (Fig. 7).

After cooling irrigation, the density of the cytoplasmic matrix of the epithelial cells was decreased and the organellae showed the tendency to be scattered. This alteration might be caused by the accumulation of fluid in the cytoplasm and did not indicate the decrease of the organellae. Therefore, this alteration may be regarded as edema of the epithelial cell. Some mitochondria were moderately swollen but the nucleus, polypoid process, basal infolding membrane and the other organellae revealed no significant morphological changes. The dilatation of the intercellular space was not observed (Fig. 8). Occasionally, below the nucleus of the epithelial cell large vacuoles with remarkably low density were observed, and around these vacuoles small vesicles were accumulated (Fig. 9).

Capillaries in the epithelium of the choroid plexus were composed of very thin endothelial cells. The endothelial cell was clearly demonstrated to posess the endothelial pore. After cooling irrigation, swelling of the endothelial cell was occasionally noted. And pinocytic vesicles were observed in this swollen endothelial cell (Fig. 10).

In the cases injected of P. V. P. in the ventricular cavity following cooling irrigation, the following findings were frequently observed; the intercellular space was dilated about 4000 to 6000 Å or more, and the basal infolding membrane was also recognized to be dilated (5000～6000 Å) and furthermore, the density of its space was very low, suggesting of the space contained fluid substance. The cytoplasmic density of the endothelial cells which showed such findings was increased (Fig. 11, 12). Even at the portion where the intercellular space was remarkably dilated, no disconnection of the terminal bar was observed.

On the other hand, dense minute granules, which were 100 to 200 Å in diameter and presumed to be derived from P. V. P. granule, were occasionally observed on the surface of the polypoid process. These granules were also recognized on the inner surface of the pore which was made up of the invaded limiting membrane of the polypoid process into the cytoplasm. The small vesicles which might be connected with the pore contained the granules, too (Fig. 13). Furthermore, P. V. P. granules were recognized in the small vesicles at the basal part of the epithelium. On the surface of the basal infolding membrane and at the peribasal membrane region (Fig. 14).

The capillary space at the center of the choroidal epithelium showed very high density, though not uniformly. And the endothelial pore and small vesicles in the endothelial cells contained dense material which showed the same density as was observed in the capillary space (Fig. 15). These findings suggested that the P. V. P. granules which were injected in the ventricular cavity might be carried into the capillary space of the choroid plexus.

These findings, however, were not observed when P. V. P. were injected in the ventricle without previous cooling irrigation.

DISCUSSION

In 1885, EHRLICH[12] observed that certain aniline dyes, when injected into the blood stream, appeared to stain all tissues of the body except those of the central nervous system; and ever since then, this phenomenon of apparent reluctance in varying degree to pass from blood to brain has been notified for many other substances, and attributed to a "blood-brain barrier."

For half a century after EHRLICH's original observations, interest was virtually confined to the behavior of dyes, mainly trypan blue, and other histologically identifiable substances such as ferricyanide and silver.

In earlier conceptions as to the site of the barrier, morphologist's interest was concentrated to certain structures between blood and cerebral tissue within the brain such as the capillary endothelium, perivascular space (VIRCHOW-ROBIN space) and the pia-glia membrane, regarded as its localization, before introduction of the electron microscopical technique in this field.

As to the electron microscopical study of the capillary blood vessel in the central nervous system, van BREEMAN & CLEMENTE[35], DEMPSEY & WISLOCKI[8], LUSE[19], MAYNARD, SCHULTZ & PEASE[21] & DONAHUE & PAPPAS[11] reported almost the same observation; The capillary is consisted of thin endothelial cells which is completely encircled by the homogeneous dense basement membrane measuring from 100 to 500 Å in width, and its outer portion is supported by the glial processes. VIRCHOW-ROBIN's space which has been observed by the light microscopy is not demonstrated electron microscopically. On the other hand, the fine structure of the capillary in the other organs is almost similar to that recognized in the central nervous system. But the "barrier" function is not recognized in any places except in the central nervous system. In the organs other than the central nervous system, there is a pericapillary space where a few fibroblasts and collagen fibers interpose. Outside of this space, there exist the parenchymal cells. It is not so difficult to suppose that these morphological differences bring about the differences in functions such as the transportation of fluid, oxygen, glucose and others. In most organs, the pericapillary space is filled with the tissue fluid, but in the cerebrum, the glial cytoplasm is interposed between the capillary wall and the cerebral substance. So as to the transportation of a certain substance to the parenchymal cell, tissue fluid plays a significant role in case of most organs, but on the other hand, in case of the central nervous system, the glial cytoplasm participates in such function. If one takes account of the existence of the cytoplasmic membrane and mitochondria which regulate the exchange of ion and molecules and contain much enzyme respectively, differences in transportation of substances may be easily understood.

FARQUHAR and HARTMAN[13] reported that the capillary in the central nervous system was surrounded by one or more astroglial processes, and the substance which got out of the capillary wall diffuses along the glial process and then was transported into the other cells. On the basis of the electron microscopical findings, GERSCHENFELD[15] et al. also

described that the astroglial cells interposed among the blood, cerebrospinal fluid and nerve cells, and not only stored water and ion but also took charge of the exchange of substances. From above findings, the glial process in contact with the outer surface of capillary endothelium has become to be infered as a site of blood-brain barrier recently.

About the glial processes, which exist in the pericapillary space and have low density, FARQUHAR[13], SCHULTZ[29] and DE ROBERTIS[7] regarded them to be the astrocyte, but on the other hand, LUSE[19][20] considered them to be oligodendroglia. These glial processes, however, are so identical in their appearance that their opinions were only opposite as to the identification of the glial cell.

On the problem of cerebral edema and cerebral swelling, SPATZ[30] reported that in case of cerebral edema cerebral surface was moistly convoluted and soft in consistency, but in case of cerebral swelling the cerebrum was rather dehydrated and showed increased consistency macroscopically. REICHHARDT[27] described that cerebral edema indicated the increased free water in the interstitial space, and cerebral swelling implies swollen intercellular substance which combined abnormally with interstitial fluid, so these two pathological changes were essentially different. Electron microscopical observation of the central nervous system reveales that the glia, nerve cell and their cytoplasmic component such as the processes (neuropile) are closely packed, and among these cellular components intercellular space measuring about 100 to 200 Å in diameter are recognized.

Though the intercellular space is regarded to have some relation with transportation of the fluid, it accounts for only one or two per cent of the whole cerebral volume. Therefore, fluid storage in the brain must be studied more in detail. Studying experimental cerebral edema by electron microscope, LUSE and HARRIS[20], TORACK, TERRY and ZIMMERMAN[33][34], and ISHII[17] confirmed that there was no dilatation of the intercellular space and markedly increased volume of the glial process, especially at the vicinity of the capillary blood vessel was prominent. From above results they pointed out that cerebral edema and its swelling might be caused by the same process and they only differed in severity. KLATZO, PIRAUX and LASKOWSKI[18] reported that cooling brought about localized cerebral edema indirectly. TORACK, TERRY and ZIMMERMAN[33], injecting trypan blue in the mouse which was previously exposed carbon dioxide ice on the surface of the brain for 20 to 30 minutes, demonstrated that blood-brain barrier was locally destructed. At the same time they clarified electron microscopically that the cytoplasm of the glia cells were increased in volume. But they did not describe how were these changes brought about.

However, their observation was confined the effect of freezing of the brain but not that of mild hypothermia. In the previous studies of the cooling ventricular irrigation, morphological change of the brain has not been expected on the basis of physiological characteristics of the experimental results. Although electron microscopical findings of the ependymal tissue after cooling irrigation demonstrates slight cerebral edema in this experiment, these changes does not imply so severe destruction as physiological function involved in the tissue might abolish.

So far as the slight cerebral edema observed after the cooling irrigation is concerned, it is unable to determine whether mere effect of local hypothermia or hydrodynamic effect of irrigating fluid is responsible for the initiation of it.

On cerebrospinal fluid brain barrier, DRASKOCI[9] et al. injecting histamine into the ventricular cavity, observed that this substance penetrated into the third ventricular wall attaining a depth of 2.5 cm in an hour. FERDBERG and FLEISCHHAUSER[14] injected bromphenol blue into the ventricle, but this substance did not penetrate the ventricular wall. They considered that the ependymal cells might play a significant role in transportation of substances from cerebrospinal fluid to the cerebral substance. Function of this barrier, however, still remains obscure and further studies are necessary. BRIGHTMAN[1)2)3)] studied the transportation of ferritin particles injected into the ventricular cavity of rat. According to his observation, although a few particles passed through the intercellular space, most of them were observed to reach to the capillary endothelial cell and capillary space under the ependymal cell passing through the large vacuoles and vesicles in the ependymal cytoplasm. But he did not mention which plays more significant role as barrier, the ependymal cell or the neuropile in the ventricular wall. In the present experiment, the ependymal cell revealed no significant changes, but this does not mean that the ependymal cell holds the key to the problem of barrier. Further studies into this problem are necessary.

The role of the choroid plexus in production of cerebrospinal fluid has been studying for a long time from anatomical, histological and biochemical standpoints. But its definite significance has not been assessed. According to the "secretion theory", the choroid plexus may secretes cerebrospinal fluid actively. CUSHING[6] observed directly cerebrospinal fluid flowing out from the choroid plexus, and DANDY[10] reported that the choroid plexus played an important part in production of this fluid on the basis of experimental study. Since then, this theory has been supported by many investigators. DEMPSEY and WISLOCKI[5], van BREEMAN and CLEMENTE[35], MILLEN and ROGERS[23], MAXWELL and PEASE[22], SHRYOCK and CASE[31] and NAKANISHI[25] examined the choroid plexus by electron microscope. The polipoid process and basal infolding membrane are appeared as the characteristic structures in the cytoplasm of the epithelium of the choroid plexus. MAXWELL and PEASE[22] regarded these structures to have close relation to the production of cerebrospinal fluid, because the basal infolding membrane of the choroid plexus is quite similar to that of the tubular epithelium in the kidney (this finding was first reported by PEASE[27]) and of the epithelial cells in the salivary gland, and ciliary body which are closely related to fluid metabolism. NAKANISHI[25] also accepted their review. The polypoid processes, on the other hand, is encircled by the limiting membrane and is supposed to contain abundant fluid substance because of its round shape and remarkably low density. MAXWELL and PEASE[22] pointed out that the difference in the size of this process might have some relation to the secretion of cerebrospinal fluid. van BREEMAN and CLEMENTE[35] also referred the significance of the polypoid process in the production of cerebrospinal fluid. However, there is no convicting evidence to support their assumption. In the present experiment, the polypoid process varied in size and length. Morphologically, the author could not clarify if the polypoid process has any intimate relation with the secretion of cerebrospinal fluid and what kind of changes are brought about to the process by hypothermia. In view of the electron microscopical picture the epithelial cells in the choroid plexus have the ground substance with moderate density, and between these cells the intercellular basal layer interposes. The similar findings are noted also in the tubular epithelium of the kidney, this

layer was named as "cement layer" by PEASE[27]. On the other hand, characteristic endothelial pore is recognized in the capillary endothelium of either the kidney or the choroid plexus. As is described by NAKANISHI[25], the basement membrane of capillary has the basement pore, which may enable the plasma or other substances to penetrate into the intercellular space. Intercellular space of the choroid plexus is described as markedly wide space containing much fluid, and the fibrocyte, collagen fibril and neurofibril are scatteringly recognized there. DEMPSEY and WISLOCKI[8] and VAN BREEMAN and CLEMENTE[35] examined the choroid plexus of the rat which had been administered with 0.5 per cent aqueous solution of silver nitrate in drinking water over a period of 6 to 8 months, and they recognized the silver particles accumulating in the basal membrane and basal limiting membrane.

From this finding they suggested that blood cerebrospinal fluid barrier might exists in the basement membrane and intercellular basal layer of the choroid plexus.

After cooling irrigation, the matrix of the epithelial cell of the choroid plexus showed decreased density and looked "watery", just as fluid accumulated in the cytoplasm. At the same time, the endothelial cell of capillary was also swollen. From these findings, it is possible to speculate that fluid component reaches to the capillary space, easily passing through the cellular limiting membrane and basement membrane. It is generally accepted that the permeability of the cell is depend on its state and temperature. Therefore, giving careful consideration to the increased permeability and hypofunction of the choroid plexus due to hypothermia, it may be more reasonable to presume that accumulated fluid was derived from irrigating fluid in the ventricle through the choroidal epithelium. The significance and true nature of the large vesicles below the nucleus are still remain obscure. When the transportation of fluid is discussed, the osmotic pressure of the extracellular fluid must be considered. But it may be beside the question in this experiment, because isotonic Ringer's solution was used.

Transportation of fluid component from the ventricle to the capillary as the result of hypothermia was further studied by injecting P. V. P. granules into the ventricular cavity after cooling irrigation.

When P. V. P. was infused into the ventricle, markedly dense granules of P. V. P. were recognized characteristically on the surface of the polypoid process. Furthermore, these granules were observed to appear on the basal infolding membrane, small vesicle and apical pore, and further penetrated into the capillary space through the endothelial pore of the capillary wall. These observations might suggest that P. V. P. solution pass through these cellular organellae. In other words the fluid and colloid particle in the ventricle are seemed to be transported into the blood capillary space. Above mentioned findings were not obtained when P. V. P. solution was infused into the ventricle without previous cooling irrigation. This implys that the increased permeability of the epithelial cellular membrane and its basement membrane in the choroid plexus is due to the cooling irrigation. When P. V. P. solution was infused into the ventricle after cooling irrigation, the basal infolding membrane and intercellular layer of the epithelial cell were both dilated. And the matrix of the epithelial cell showed increased density, which might suggest that cellular fluid was stored in this space and the cytoplasm became rather dehydrated.

Above mentioned findings following cooling irrigation of the ventricle remain to be determined whether these changes are temporary or continue for a long time causing cellular damage.

SUMMARY

In order to determine the morphological effect of cooling irrigation of the cerebral ventricles with cold Ringer's solution upon the periventricular nervous tissue, electron microscopical study was performed on the ependymal tissue following the cooling irrigation.

Although only slight edema was observed within above tissue, cooling ventricular irrigation lasting as long as an hour did not seem to damage essentially such an important area for the maintenance of responsiveness as close to the ventricular wall.

After the cooling irrigation, edematous swelling was also observed in the epithelial cell of the choroid plexus. Polyvinyl pyrrolidon granules infused into the ventricular cavity following the cooling irrigation were appeared to penetrate the epithelium into the capillary, while those infused without previous cooling irrigation did not be demonstrated by electron microscope. From these findings, it might be presumed that the permeability of the epithelial basal membrane of the choroid plexus was increased to allow penetration of the granules as the result of the cooling irrigation, in connection with the water transport mechanism involved.

The author is indebted to Prof. SHUJI HOSOKAWA and Assistant Prof. FUMIYA UCHINO the Department of Pathology in our University, for kind guidance and technical advice throughout this investigation.

REFERENCES

1) Brightman, M. W. and Palay, S. L.: The fine structure of ependyma in the brain of the rat. J. Cell. Biology, **19**: 415-439, 1963.
2) Brightman, M. W.: The fine structure of ciliated ependyma (abstract). Anat. Rec., **139**: 210-211, 1961.
3) Brightman, M. W.: An electron microscopic study of ferritin uptake from the cerebral ventricles of rats (abstract). Anat. Rec., **142**: 219, 1962.
4) Browan, T.: The permeability of the cerebral vessels in normal and pathological condition. Copenhagen, Munksgaaod, 1949.
5) Caulfield, J. B.: Effects of varying the vesicle for OsO_4 in tissue fixation. J. Biophysic. & Biochem. Cytol., **3**: 827-829, 1957.
6) Cusching, H.: Study on the C. S. F. B. Med. Research, **31**: 1-19, 1911.
7) De Roberts, E., Gerschenfeld, H. M. and Wald, F.: Cellular mechanism of myelination in the central nervous system. J. Biophysic. & Biochem. Cytol., **4**: 651-656, 1958.
8) Dempsey, E. W. and Wislocki, G. B.: An electron microscopic study of the blood-brain barrier in the rat, employing silver nitrate as a vital stain. J. Biophysic. & Biochem. Cytol., **1**: 245-256, 1955.
9) Draskoci, M., Felberg, W., Fleuschhauer, K. and Haranath, P. S. R. K.: Absorption of histamin into the blood stream on perfusion of the cerebral ventricles and its uptake by brain tissue. J. Physiol., **150**: 50, 1960.
10) Dandy, W. E.: Experimental study by hydrocephalus. Ann. Surg., **70**: 122-144, 1919.
11) Donahue, S. and Pappas, G. B.: The fine structure of capillaries in the cerebral cortex of the rat at various stage of development. Am. J. Anat., **108**: 331-347, 1961.
12) Ehrlich, P.: Das Sauerstoffbedürfnis des Organismus. In: Eine Farbenanalytische Studie. Berlin: 1885.
13) Farquhar, M. G. and Hartman, J. F.: Neuroglial structure and relationship. J. Neuropath. & Exper. Neurol., **16**: 18-39, 1957.
14) Feldberg, W. and Fleischhauer, K.: Penetration of bromophenol blue from the perfused cerebral ventricles into the brain tissue. J. Physiol., **150**: 451-462, 1960.

15) Gerschenfeld, H. M., Flora Wald, J. A. Zadunaisky and E. D. P. De Robertis: Function of astroglia in the water-ion metabolism of the central nervous system. An electron microscope study. Neurologie, **9**: 412-425, 1959.
16) Higashi, K.: Experimental studies on the cooling irrigation of cerebral ventricular system (I), (II). Arch. Jap. Chir., **26**: 624-649, 1957.
17) Ishii, S.: Electron microscopic studies on B. B. B. in cerebral swelling. Brain & Nerve, **14**: 357-361, 1962.
18) Klatzo, I., Piraux, A. and Laskowski, E. J.: The relationship between edema, blood-brain barrier and tissue elements in a local brain injury. J. Neuroph. & Exper. Neurol., **17**: 548-564, 1958.
19) Luse, S. A.: Electron microscopic observations of the central nervous system. J. Biophysic. & Biochem. Cytol., **2**: 531-542, 1956.
20) Luse, S. A. and Harris, B.: Electron microscopy of the brain in experimental edema. J. Neurosurg., **17**: 439-446, 1960.
21) Maynard, E. A., Schultz, R. L. and Pease, D. C.: Electron microscopy of the vascular bed of rat cerebral cortex. Am. J. Anat., **100**: 409-433, 1957.
22) Maxwell, D. S. and Pease, D. C.: The electron microscopy of the choroid plexus. J. Biophysic. & Biochem. Cytol., **2**: 467-474, 1956.
23) Millen, J. W. and Rogers, G. E.: An electron microscopic study of the choroid plexus in the rabbit. J. Biophysic. & Biochem. Cytol., **2**: 407-416, 1956.
24) Millen, F. W. and H. Hess: The blood-brain barrier. An experimental study with vital dyes. Brain, **81**: 248-257, 1958.
25) Nakanishi, A.: Electron microscopic studies on the ultrastructure of the choroidplexus. J. Juzen Med. Soc., **63**: 460-483, 1959.
26) Pease, D. C.: Electron microscopy of the vascular bed of the kidney cortex. Anat. Rec., **121**: 701-722, 1955.
27) Pease, D. C.: Electron microscopy of the tubular cells of the kidney cortex. Anat. Rec., **121**: 723-743, 1955.
28) Reichhardt, M.: Hirnschwellung. All. Z. Psychiat., **75**: 31-103, 1919.
29) Schultz, R. L., Maynard, E. A. and Pease, D. C.: Electron microscopy of neurons and neuroglia of cerebral cortex and corpus callosum. Am. J. Anat., **100**: 369-407, 1957.
30) Spatz, H.: Die Bedeutung der "Symptomatischen" Hirnschwellung für die Hirntumoren und für andere raumbeengende Prozesse in der Schädelgrube. Arch. Psychiat., **88**: 790-791, 1929.
31) Shryock, E. H. and Case, N. M.: Light and electron microscopy of the choroid plexus in dog. Anat. Rec., **124**: 361, 1956.
32) Tokuoka, S. et al.: Cooling irrigation of the cerebral ventricular system. Yamaguchi-Igaku, **11**: 107-116, 1962.
33) Torack, R. M., Terry, R. D. and Zimmerman, H. M.: The fine structure of cerebral fluid accumulation. Am. J. Path., **35**: 1135-1147, 1959.
34) Torack, R. M., Terry, R. D. and Zimmerman, H. M.: The fine structure of cerebral fluid accumulation. Am. J. Path., **36**: 273-287, 1960.
35) van Breeman, V. L. and Clemente, C. D.: Silver deposition in the central nervous system and the hematoencephalic barrier studied with the electron microscope. J. Biophysic. & Biochem. Cytol., **1**: 161-166, 1955.
36) Wislocki, G. and Ladman, A. J.: The demonstration of a blood-ocular barrien in the albinorat by means of the intravitam deposition of silver. J. Biophysic. & Biochem. Cytol., **1**: 501-509, 1955.

LEGENDS FOR FIGURES

Figures 2 to 15 are electron micrographs.

Key:

BIM : basal infolding membrane
BV : blood vessel
CF : collagen fibril
ECN : epithelial cell nucleus
ER : endoplasmic reticulum
M : mitochondria
PP : polypoid process
VC : ventricular cavity
BM : basement membrane
CGN : clear glial cell nucleus
EC : endothelial cell
EN : ependyma cell nucleus
GP : clear glial processes
M' : swelling of mitochondria
RBC : red blood cell

和文抄録

脳室灌流冷却に関する実験（第3報）

—脈絡叢及び脳室壁近傍の電顕像—

山口県立医科大学第2外科学教室

中　村　輝　美

我々の教室では，1955年以来，中枢神経系の遮断を目的として，冷リンゲル氏液で，脳室系を灌流冷却することにより，動物が迅速且つ可逆性の unresponsiveness を来すことを確認した．著者は脈絡叢及び脳室壁近傍の形態学的変化について電子顕微鏡的に観察したので報告する．

実験動物は1～7kgの成犬を用い一側の側脳室より大槽に至る脳室系を，冷リンゲル氏液で灌流後固定液（Caulfield の処方）5 ml を脳室腔に注入して，脳室壁およびその近傍を固定後直ちに脳を摘出し，脈絡叢および脳室附近の組織を試料とした．

脳室灌流前後の脳室附近の毛細血管およびその周囲のグリア突起の微細構造の電顕像では，灌流後には脳浮腫の際にみられる様な毛細血管周囲のアストログリア突起の重脹を認めるが，かかる所見を呈する部位は比較的少なく，又その程度も軽く灌流冷却後の電顕像での変化は軽微であると考えられる．

他方脈絡叢上皮細胞では，細胞質の matrix の density が低下し，介在する Organellae は散在し，又 large vacuole を認めることもある．即ち，上皮細胞は浮腫を起したものと思われる．しかし髄液産生に重要な細胞内微細構造であると考えられている basal infolding membrane の著変は認められない．

冷リンゲル氏液で灌流後 polyvinyl pyrrolidon を脳室腔へ注入した実験例では，灌流冷却後に認めたような浮腫像を呈せず polyp 状突起の周辺に P. V. P. 顆粒と考えられる微細顆粒が附着している．この細顆粒は又上皮細胞内の小胞体や basal infolding membrane 内にも認められ，更に脈絡叢血管腔内にも認められるが，灌流冷却を行なわず，P.V.P. のみを脳室腔に注入した実験群ではかかる所見は認められず，このことからは，脳室灌流が，髄液の血中への移行に何らかの影響を及ぼしていると考えられる．

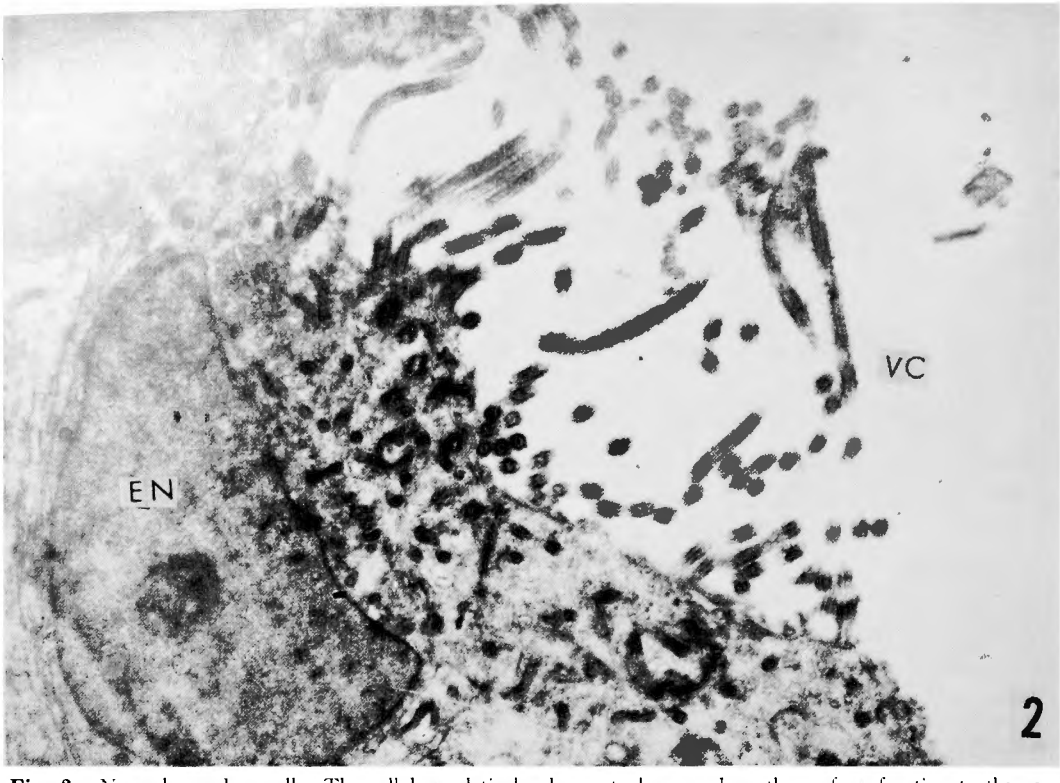


Fig. 2. Normal ependym cell. The cell has relatively clear cytoplasm, and on the surface fronting to the ventricular cavity the ciliary shafts are seen (× 18400).

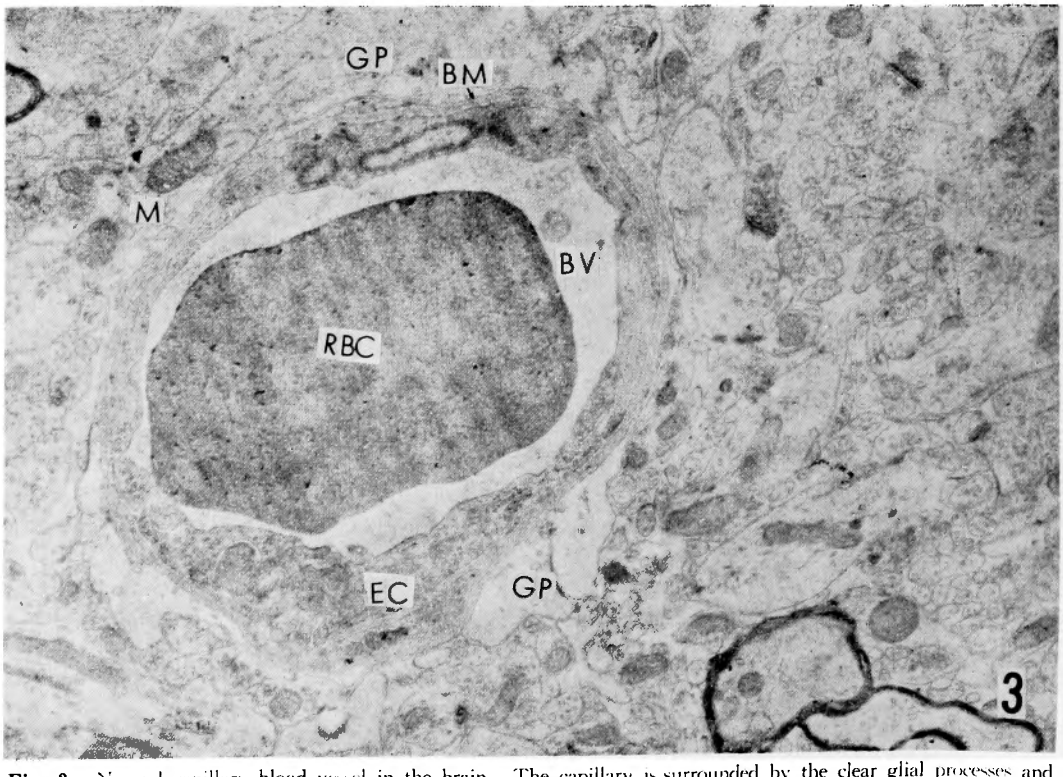


Fig. 3. Normal capillary blood vessel in the brain. The capillary is surrounded by the clear glial processes and no space is observed between the basal membrane of the blood vessel and the cytoplasm of the glia (× 14000).

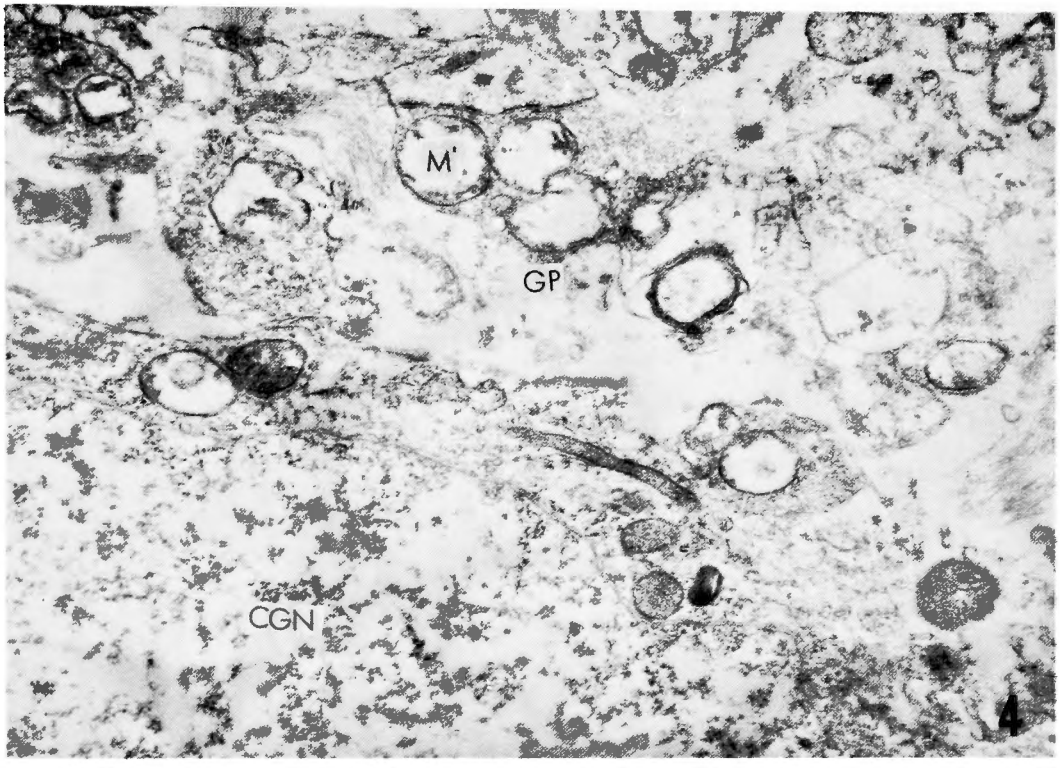


Fig. 4 The cerebral tissue near the lateral ventricular cavity after cooling irrigation. The clear glial process reveals decreased density. The right portion show fine fibrils (× 18400).

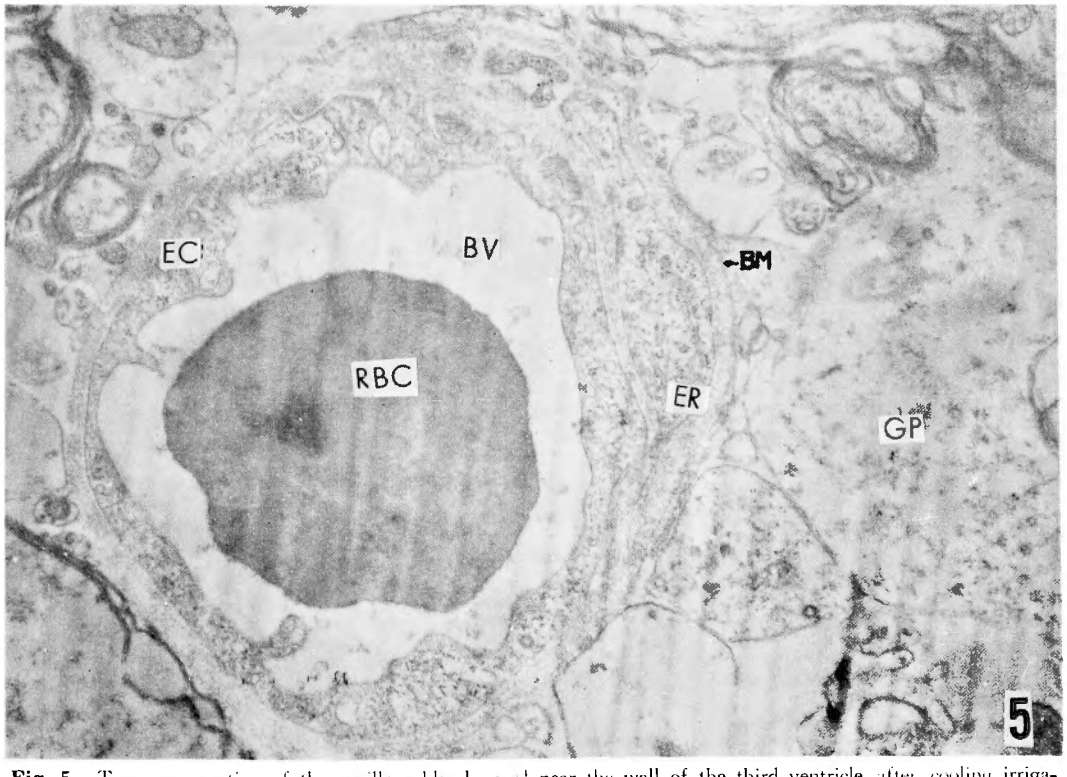


Fig. 5. Transverse section of the capillary blood vessel near the wall of the third ventricle after cooling irrigation. Glial processes around the blood vessel show increased volume with resultant markedly decreased density. In the endothelial cells, the endoplasmic reticulum is increased in number and is dilated (× 20000).

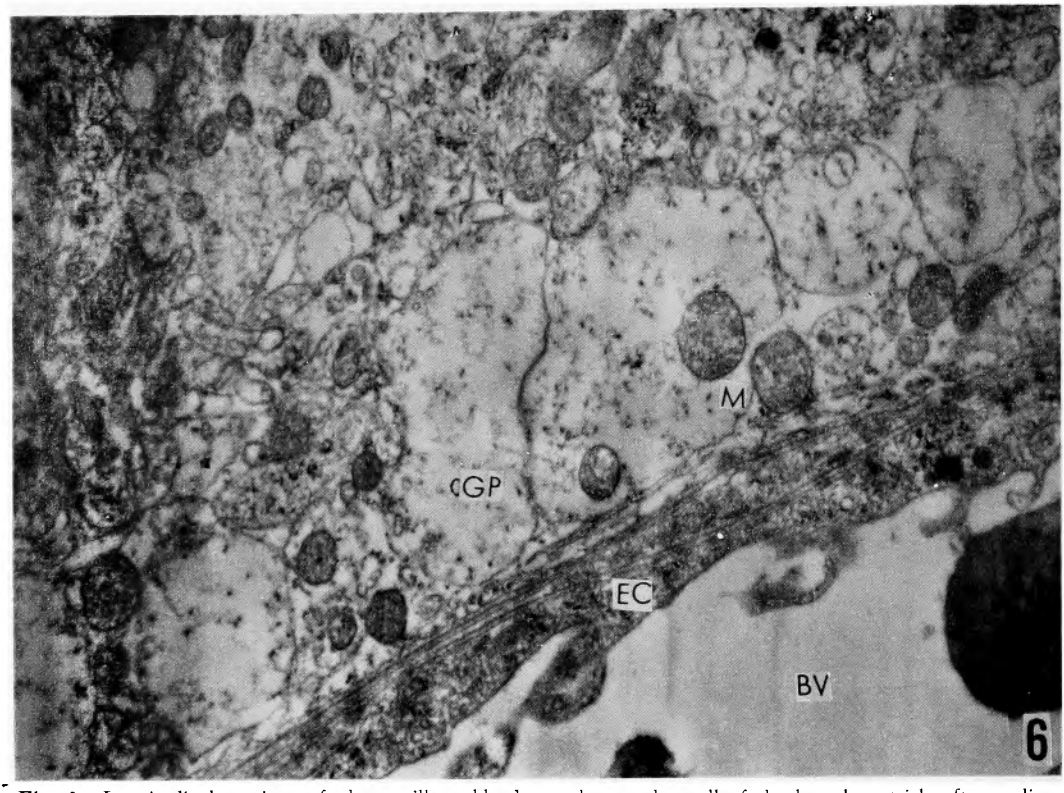


Fig. 6. Longitudinal section of the capillary blood vessel near the wall of the lateral ventricle after cooling irrigation. The volume of the glial process is slightly increased and around the basement membrane, swollen mitochondria are observed (× 18400).

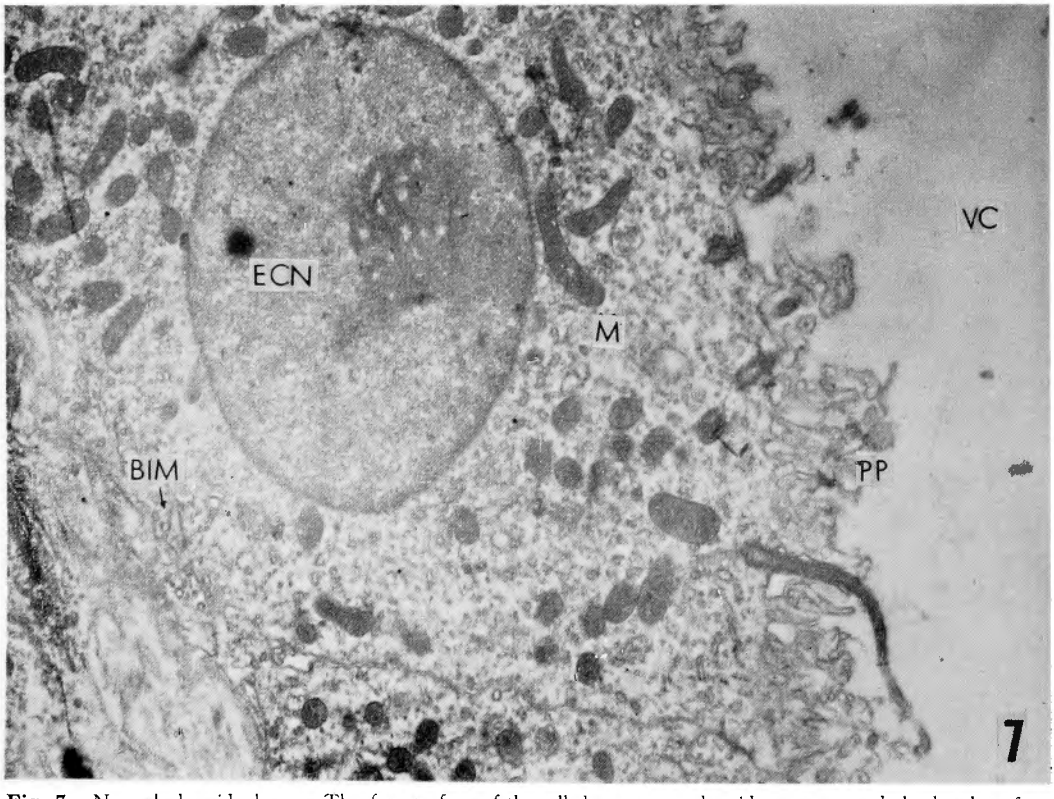


Fig. 7. Normal choroid plexus. The free surface of the cells has many polypoid processes and the basal surface is provided with numerous basal infoldings (× 11500).

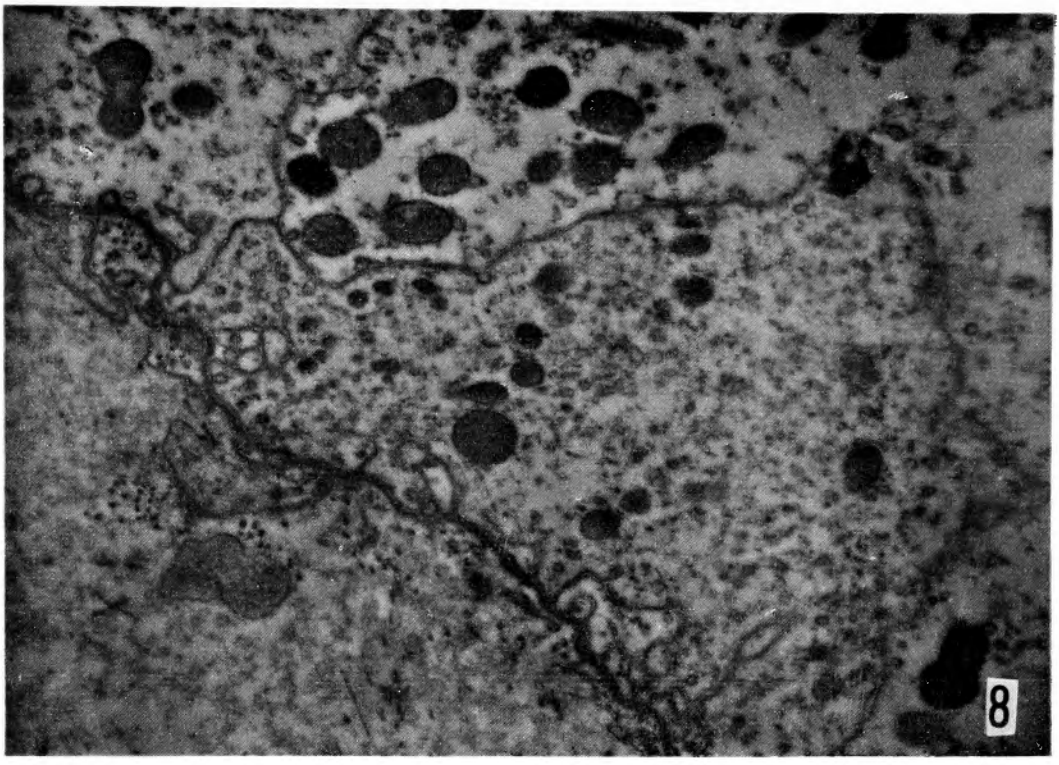


Fig. 8. The epithelium of the choroid plexus after cooling irrigation. The cytoplasmic matrix reveals decreased density. Especially the cells at the left and upper portion show remarkable changes, and the organellae are scatteringly seen (× 11500).

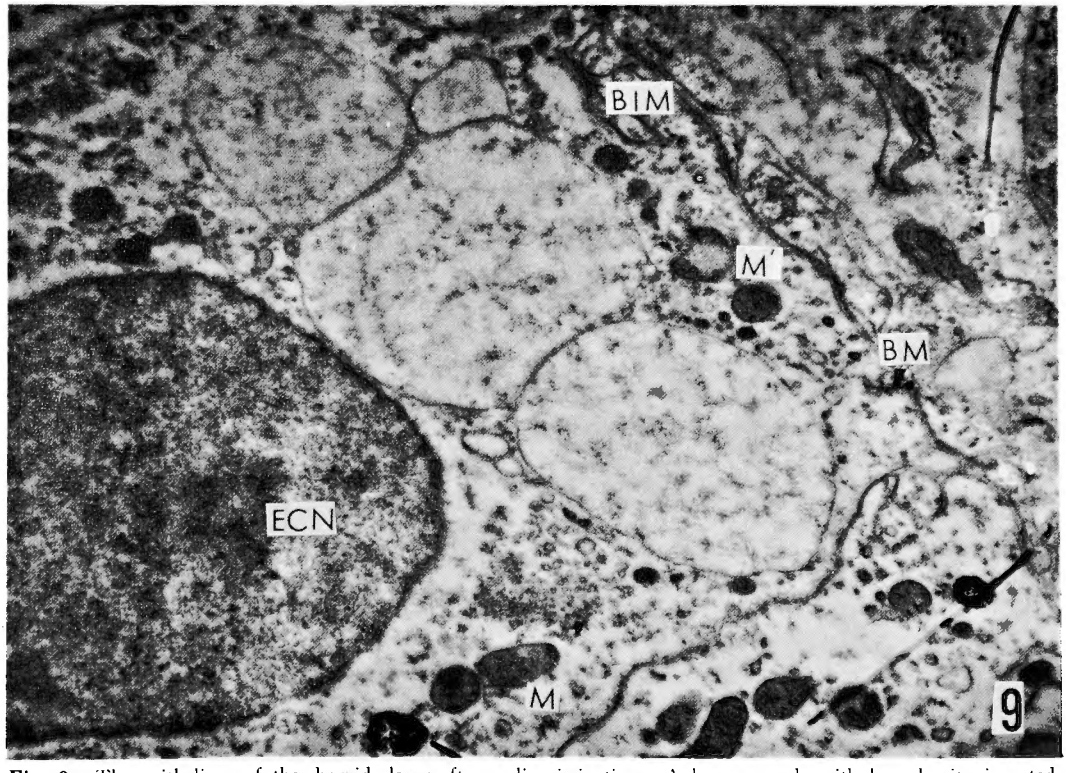


Fig. 9. The epithelium of the choroid plexus after cooling irrigation. A large vacuole with low density is noted at the vicinity of the nucleus (× 15000).

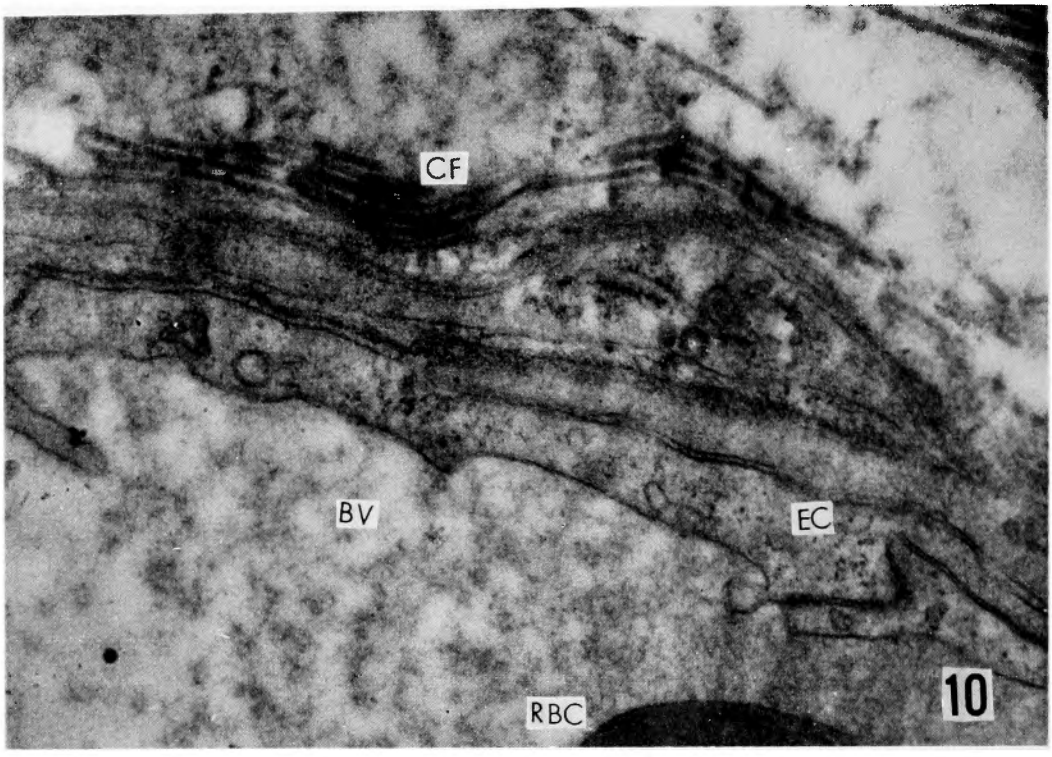


Fig. 10 The capillary blood vessel in the choroid plexus after cooling irrigation. The endothelial cells show increased volume and the endoplasmic reticulum is also increased in number (× 30000).

Fig. 11. Transverse section of the choroid plexus after injection of P. V. P. solution into the ventricle following previous cooling irrigation. Three epithelial cells are observed. The intercellular space (arrow) is dilated ranging from 4000 to 6000 A or more (× 15000).

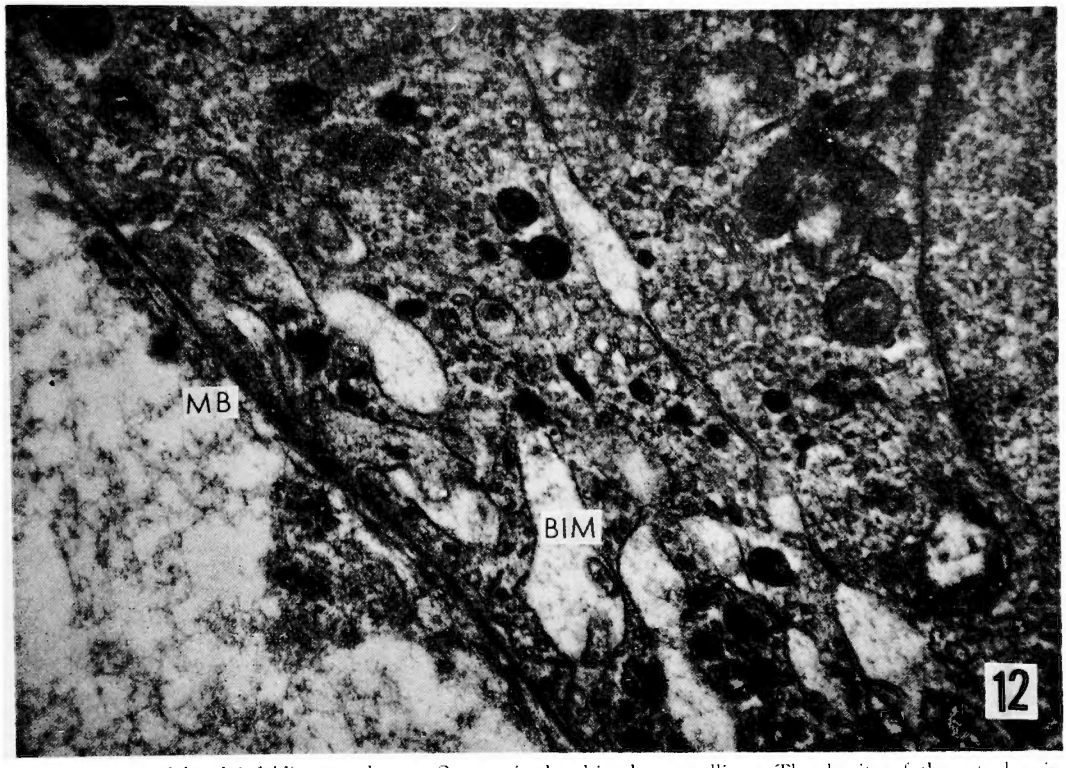


Fig. 12. Dilated basal infolding membrane. Some mitochondria show swelling. The density of the cytoplasmic matrix is increased (× 16100).

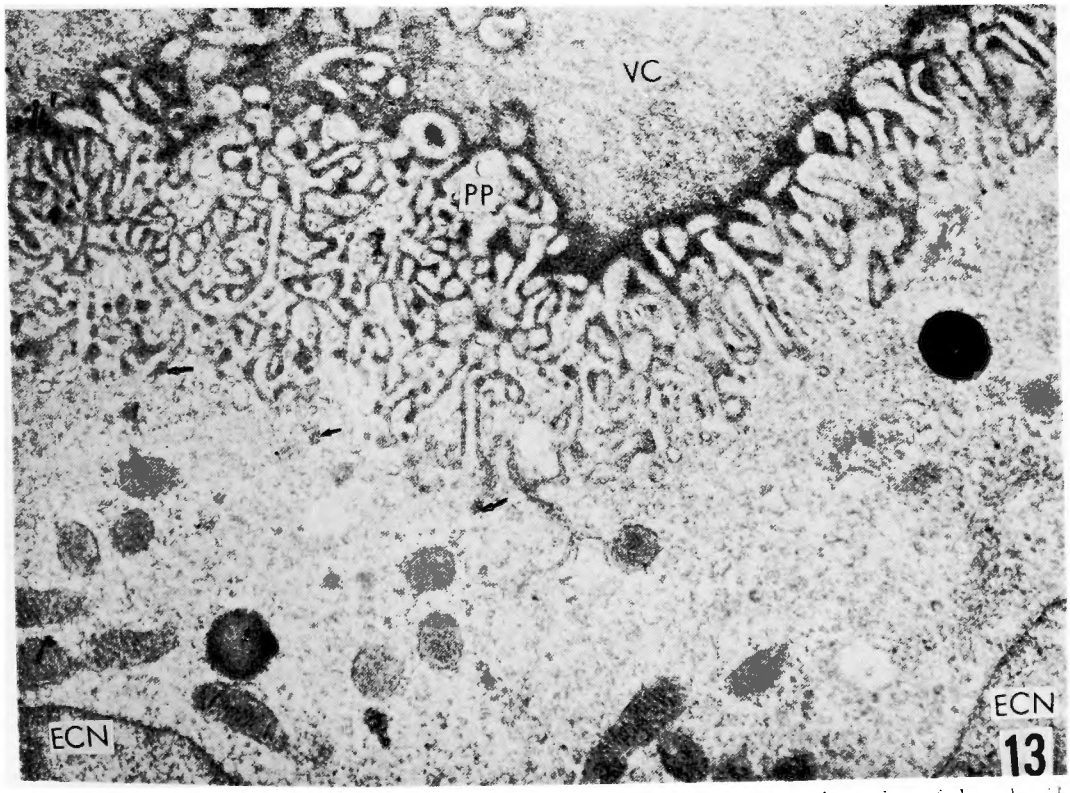


Fig. 13. The minute granules (100~200 Å) with high density are recognized on the surface of the polypoid processes. These granules are also seen in the small vesicles near the polypoid processes (arrow) (× 15000).

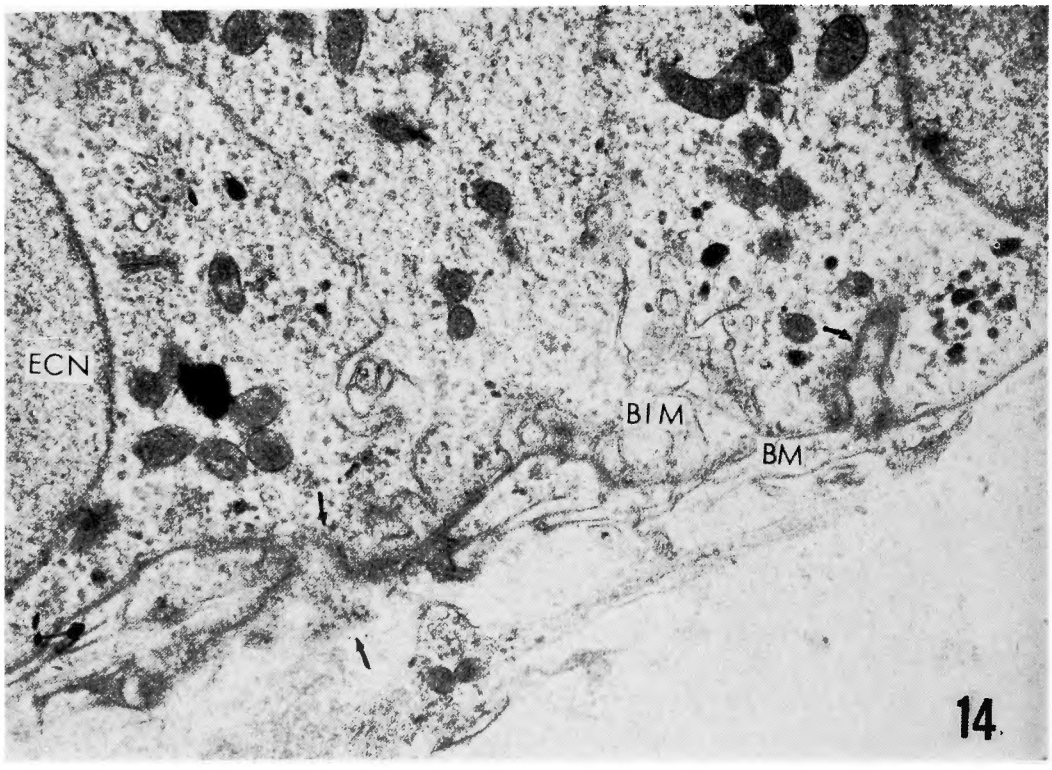


Fig. 14. The minute granules in the small vesicles and basal infolding membrane, many granules are observed especially at the vicinity of the basal membrane (arrow) and they are also seen in the pericapillary space (× 15000).

Fig. 15. The minute granules in the endothelial pore (arrow). The capillary space is packed with these granules (× 15000).